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## ARCHITECTONICS OF THE HAIRS OF THE WOOLLY MAMMOTH AND WOOLLY RHINO

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### ABSTRACT

SEM studies of hairs of two individuals of the woolly rhinoceros (rhino) *Coelodonta antiquitatis* and six individuals of the woolly mammoth *Mammuthus primigenius*, and hairs of matted wool (“wads”) of a possible woolly mammoth and/or woolly rhinoceros (X-probe) showed that coloration and differentiation of the hair, hair shaft shape, cuticle ornament and cortical structure are similar in both species and in the X-probe. The cortex has numerous longitudinal slits, which some authors misinterpret as medullae. In both species, the medulla is degenerative and does not affect the insulation properties of the hairs. Nevertheless its architectonics, occasionally discernible in thick hairs, is a major diagnostic for identification of these species. The hair structure of rhino is similar to that of the vibrissae of some predatory small mammals and suggests increased resilience. The X-probe contained numerous the woolly mammoth hairs, a few hairs of the woolly rhino and ancient bison *Bison* spp. The morphological identification of these mammals hairs is confirmed by genomic sequencing. The multi-layered long fur (not the architectonic of hairs) was a major adaptation of the woolly mammoth and woolly rhino to a cold climate.

**Key words:** architectonics, genomic sequencing, hair, identification, woolly mammoth, woolly rhino

## АРХИТЕКТОНИКА ВОЛОС ШЕРСТИСТОГО МАМОНТА И ШЕРСТИСТОГО НОСОРОГА

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### РЕЗЮМЕ

Изучение методом СЭМ волос двух особей шерстистого носорога *Coelodonta antiquitatis* и шести особей шерстистого мамонта *Mammuthus primigenius*, и волос из колтунов неизвестной шерсти, возможно, принадлежавшей мамонту и/или носорогу (Х-проба) показало, что окрас и дифференциация волосяного покрова, конфи-

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гурация стержня волос, орнамент кутикулы и строение коркового слоя сходны у обоих видов и у X-пробы. Кора имеет многочисленные продольные трещины, которые некоторые авторы интерпретируют как сердцевину. У обоих видов сердцевина дегенеративная и не влияет на теплозащитные свойства волос. Тем не менее, ее архитектоника, иногда присутствующая в толстых волосах, является основным диагностическим признаком для идентификации этих видов. Структура волос носорога сходна с таковой у вибрисс некоторых мелких хищных млекопитающих и служит для повышения прочности волос. X-пробы содержат многочисленные волосы мамонта, единичные волосы носорога и древнего бизона *Bison* spp. Морфологическая идентификация волос этих млекопитающих подтверждена геномным секвенированием. Основной адаптацией к холодному климату у мамонта и носорога служила многослойная шерсть, а не архитектоника волос.

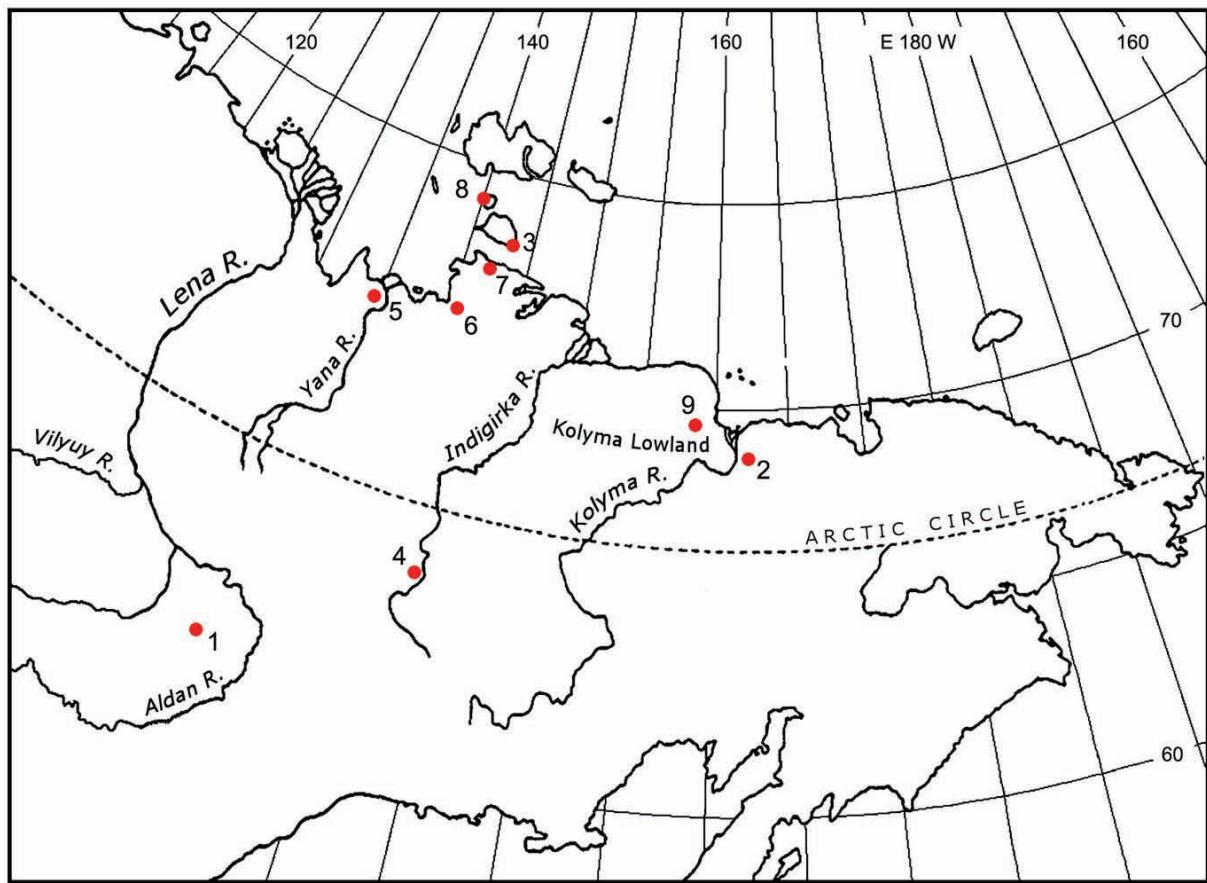
**Ключевые слова:** архитектоника, геномное секвенирование, волосы, идентификация, шерстистый мамонт, шерстистый носорог

## INTRODUCTION

The study of the hair of the woolly mammoth *Mammuthus primigenius* (Blumenbach, 1799) has a long history. At the beginning of the 20th century it was shown that the woolly mammoth's body was covered with hair, and that it possibly had a mane (Zalenskiy 1909). Judging from old petroglyphs and Palaeolithic figurines, the woolly mammoth had a mane of long hair from the temple along the back covering the shoulders and forming a long "skirt" on either side of the body, as in the yak. The tail terminated in a tuft of long hair, as in the elephant, but the tuft was longer and thicker. Some paleontological descriptions of later woolly mammoth occurrences mention the colour and length of the hair covering the body, usually partly preserved on the body or fallen from it. For example, the Oimyakon baby mammoth (Fig. 1) had reddish-brown hair, and in the Yuka puberty-age mammoth hair colour is different in different parts of the body (Table 1). On the body, the hair is gray- and dark-brown, and reddish on the legs. In "Yuka", the maximum length of some bundles of hair on the thighs is 40–42 cm. Some hairs and small bundles reach 20 cm in length on the body, 10–14 cm on the shoulders, and 4–6 cm on the sacrum (Boeskorov et al. 2013). Möbius (1892) and Zalenskiy (1909) were the first to give morphological descriptions of the woolly mammoth hair. Möbius (1892) recognised the guard and downy hairs, whereas Zalenskiy (1909) studied the hair of the Berezovka mammoth (found in Russia in 1901), and recognised guard hairs, underhairs (or underfur), and bristle tail hairs. The tail hairs are darker, thicker and more strongly flattened than the hairs of the shoulder and abdominal skirt. Later hairs of other specimens of various ages were also partly studied (Table 1).

The woolly mammoth pelage is relatively well differentiated, in at least three tiers. The downy hairs are most numerous, constituting a dense underfur, whereas long guard hairs are less numerous, and overhairs the least numerous. The reconstruction of the woolly mammoth skin histology shows that all these hairs did not form fascicles, but grew in the skin individually and had sebaceous glands (Sokolov and Sumina 1981a; Repin et al. 2004). The structure of the overhairs of the Karginyakh baby mammoth (geological age 39000–44000 BP) was compared with that of the extant Asian elephant *Elephas maximus* Linnaeus, 1758 and the African elephant *Loxodonta africana* (Blumenbach, 1797) and was shown to differ a little in the gross structure of the overhairs and cuticle ornament (Valente 1983). However, there is evidence of specific differences of the microstructure of the tail hairs (cross-sectional shape, pigment placement, and pigment density) in African elephants, Asian elephants, and giraffes (Yates et al. 2010). The microstructure of the woolly mammoth hairs has been studied on many occasions (Hausman 1944; Sokolov and Sumina 1981a, b; Valente 1983; Tridico et al. 2014). It has been shown that only some of the overhairs have a fragmentary medulla composed of irregularly shaped cells with deeply incised margins loosely arranged along the long hair shafts. The outermost cuticle is usually strongly damaged with the free edge of its scales incised.

In the woolly rhino *Coelodonta antiquitatis* (Blumenbach, 1807) and, possibly, in the second Pleistocene genus *Dicerorhinus* Gloger, 1841, the hairs were long and dense (Kubiak and Dziurdzik 1973). This interpretation originates from the beginning of the 20th century and is based on Paleolithic petroglyphs, and on the topography of hair follicles in the skin of an adult female of woolly rhino from Starunia (West-



**Fig. 1.** Sites of recovery of the woolly rhino (WR) hair and woolly mammoth (WM) hair which were used in the present study, detailing identification details, radio carbon dated ages (if present), sex and age for each hair sample used in the present study. All samples are found on the territory of Yakutia. WR: 1 – Lena-Aldan interfluve, Churapcha settlement,  $19500 \pm 120$  years before present (GIN-9594), adult female (old); 2 – lower reaches of the Kolyma River, near Chersky settlement, Malaya Filippova River;  $39140 \pm 390$  yBP (OxA-18755), adult female (young). WM: 3 – The Bol'shoy Lyakhovskiy Island, near Shalaurov cape, adult female (?); 4 – the Ol'chan mine, Oimyakonskiy District,  $41300 \pm 900$  yBP (GrA-30727), baby female; 5 – the Kular gold mine, adult, sex unknown; 6 – near the Yukagir settlement, Ust'-Yanskiy District, about 18500 yBP, adult male (old) ("Yukagir" WM); 7 – Yogoyskiy Yar,  $34300 (+260, -240)$  yBP (GrA-53289), juvenile female ("Yuka" WM); 8 – The Maly Lyakhovskiy Island, adult female; 9 – X-probe – unknown species, WM and/or WR (F-2362/1), the Bol'shaya Chukoch'ya River.

ern Ukraine) (Starunia,  $48^{\circ}42'N$ ,  $24^{\circ}30'E$ , 1929,  $32255 \pm 775$  BP) (Kubiak and Dziurdzik 1973). The hairs from the mummified body of another adult female found in the lower reaches of the Kolyma River (Boeskorov et al. 2009), is light brown, with isolated inclusions of dark hairs. The wool of the Churapcha rhinoceros studied by ourselves is yellowish, with a few dark hairs (Fig. 2A).

No fundamental differences were found in the structure of the hairs of living and extinct rhinoceroses: in all rhinoceroses, shafts do not have a medulla, their cuticle ornament is mosaic, and the hair shaft is

regular cylindrical. All main hair structures are not species specific and do not allow identification even to genus. In living rhinoceroses, the hairs are more or less reduced. Hair follicles are present over the entire body, but the hair shaft does not extend to the surface of the skin, or is mechanically polished off, or is atrophied (Cave 1969; Chernova et al. 1998). In the African white rhinoceros *Ceratotherium simum* (Burchell, 1817) and the Indian rhinoceros *Rhinoceros unicornis* Linnaeus, 1758 hairs grow along the edge of the ears and on the tip of the tail; in the Sumatran rhinoceros *Dicerorhinus sumatrensis* (Fischer, 1814), the body is

**Table 1.** Morphological characteristics of hair of recent species of Rhinocerotidae, extinct woolly rhino and woolly mammoth (according to the literature and original data).

Reference	Species, numbers of specimens, the name and place of storage. Sex and age. Collection number. Place of storage. Geological age	Topography of probes, categories of hair, n – number of hairs studied	Width of a shaft, lim, $\mu\text{m}$	Length, mm, stiffness, coloration of fragments	Width of the medulla, % of the shaft width, lim	Shaft configuration
Recent species of Rhinocerotidae						
Ryder 1962	<i>Diceror bicornis</i>	Overhair	116–136	Straight, flexible	No data	Cylindrical, slightly curved
Cave 1969; Van den Berg 1970; Cave and Wingstrand 1972	<i>Rhinoceros unicornis</i> , <i>Rh. sondaicus</i> , <i>D. bicornis</i> , <i>Ceratotherium simum</i> , <i>Diceror sumatrensis</i>	Eyelashes	No data	Upper eyelid – thick, solid, length is 10 mm. Lower eyelid – more short and thin	No data	Cylindrical
Woolly rhinoceros						
Topography is unknown						
No. 1. Lena-Aldan interfluve, Churapcha settlement, Yakutia. Female, <i>adultus</i> . No. 2114. DPMGI SB RAS <sup>1</sup> .19350±120 y BP (GIN-9594)						
Original data						
From upper part of the hindlimb						
No. 2. Lower reaches of the Kolyma River, near Chersky settlement, Malaya Filippovka River. Female, <i>adultus</i> . No. 7938. ASY <sup>a</sup> 39140±390 y BP (OxA-18755)						
Overhair, n = 5	226–271	Rare, straight, black fragment, length – 7 cm	Fragmental, 35–43% or absent	Cylindrical		
Guard I, n = 5	113–124					
Underhair I, n = 10	56–79	Numerous, whitish fragments, length – 10 cm	Absent			
Underhair II, n = 5	34–45	Rare, whitish				
Underhair III, n = 5	11–17	Single, whitish				
From upper part of the hindlimb						
Overhair, n = 5	169–282	Reddish fragments, length – 18 cm	Fragmental, 35–52%	Cylindrical		
Guard I, n = 5	113–117					
Underhair I, n = 5	45–68	Short, reddish fragments, length – 7 cm	Absent			
Underhair II, n = 5II	23–34					

Table 1. (Continued)

Reference	Species, numbers of specimens, the name and place of storage. Sex and age. Collection number. Place of storage. Geological age	Topography of probes, categories of hair, n – number of hairs studied	Width of a shaft, lim, $\mu\text{m}$	Length, mm, stiffness, coloration of fragments	Width of the medulla, % of the shaft width, lim	Shaft configuration
Woolly mammoth						
Zalenskiy 1909	“Beryozovskiy”, the Beryozovka River, Yakutia. About 44000 yBP.	Guard Underhair Bristles	300–400 60–80 Rare, long, black	Long, light, flexible Shot, thin Absent	Present Absent	Cylindrical
Quakenbush 1909; Lang 1925; Farrand 1961	No data	Overhair Underfur	No data	Numerous, strong, thinner, slightly brown	No data	No data
Vereshchagin 1977	“Berelyokhskiy”, the Berelyokh River, North Yakutia	Guard Intermediate hair Underhair	250	Thin, soft, reddish Slightly yellow and brown, length – 1 m	No data	
Near the soles of the hind legs						
Sokolov and Sumina 1981	“Dima”, “Kirgilyakhskiy”, Magadan region. Male, <i>juvenis</i> . About 39000–44000 yBP	Overhair of some orders, n = 10 Underhair, n = 9	90–200 20–60	Long, 3–20 cm, strong, from slightly reddish to black Shot 1.3–1.6 cm, strong, light	Fragmental, rare Absent	Cylindrical or flattened with longitudinal groove, slightly curved, fusiform
Valente 1983		Overhair	No data	Yellow or brown, length – 10–13 cm,	There are 3 narrow medullar tapes into the base of the shaft	Cylindrical, with more narrow tip
Sokolov and Sumina 1982	“Yuribeiiskiy”, the Yuribei River, Gydanckiy peninsula, West Siberia. <i>Subadultus</i> . About 10000 yBP	Guide, n = 20 Intermediate hair, n = 10 Underhair, n = 20	110–320 100–180 30–60	Light brown and black, length – 7–74 cm Light reddish and brown, length – 5.5–8.5 cm Soft, light reddish, length – 1.5–3.5 cm	Fragmental Absent	Cylindrical, slightly curved
Tridico et al. 2014	6 mammoths + 1 rhino (not identified), Chukotka, Kamchatka	Overhairs Guard hair Underhair	No data	White, yellow, red and reddish, brown Colorless or dull yellow	Continuous, fragmental, 3–4 number of serious Absent	Cylindrical or flattened

Table 1. (*Continued*)

Reference	Species, numbers of specimens, the name and place of storage. Sex and age. Collection number. Place of storage. Geological age	Topography of probes, categories of hair, n – number of hairs studied	Width of a shaft, lim, $\mu\text{m}$	Length, mm, stiffness, coloration of fragments	Width of the medulla, % of the shaft width, lim	Shaft configuration
	No. 3 "Lyakhovskiy". The Bol'shoi Lyakhovskiy Island, Yakutia. U-LNRM <sup>2</sup>	Overhair, n = 10 Guard I, n = 10 Guard II, n = 10 Underhair I, n = 5 Underhair II, n = 5	226–282 79–90 56–68 34–45 11–17	Long, black fragments Long, light brown fragments Shot grey fragments	Fragmental, 27–33% Absent	Tail and a base of the tail
	No. 4. "Oimyakonskiy". The Ol'chan mine, Oimyakonskiy region, Yakutia. Female, <i>jivensis</i> . No. 7911. ASY <sup>3</sup> 41300±300 yBP (GRA-30727)	Oberhair, n = 5 Guard I, n = 10 Guard II, n = 5 Guard III, n = 5 Underhair, n = 5	158–169 113–136 79–90 56–68 17–23	Long, dark brown fragments Long, reddish-brown fragments Short, light grey fragments	Absent Fragmental, 7–9% Absent Slightly curved	Trunk (?)
	No. 5. "Kularskiy", the Kular mine, Yakutia. No. 6832. DPMGI SB RAS <sup>1</sup>	Guard I, n = 5 Guard II, n = 5 Underhair, n = 5	134–147 113–124 23–34	Long, reddish-brown fragments Shot, light grey fragments	Fragmental, pigmented, 5–6% Absent	A side of the body
	No. 6. "Yukagirskiy". The Yukagir settlement, Ust'-Yanskiy region, Yakutia. No. 7863. ASY <sup>3</sup> . About 18500 yBP.	Overhair, n = 5 Guard I, n = 5 Guard II, n = 5 Guard III, n = 5 Underhair, n = 5	226–248 113–124 68–79 45–56 23–34	Numerous, dark, black fragments Fragmental, Length – 13%	Cylindrical or flattened, sometimes with ovoid transversal section	Head (?)
	Original data				Fragmental, 15%	Cylindrical or flattened
					Absent	Curved

Table 1. (Continued)

Reference	Topography of probes, categories of hair, n – number of hairs studied	Width of a shaft, lim, $\mu\text{m}$	Length, mm, stiffness, coloration of fragments	Width of the medulla, % of the shaft width, lim	Shaft configuration	
Forearm:						
No. 6. "Yukagirskiy". The Yukagir settlement, Ust'-Yanskiy region, Yakutia. No. 7863. ASYa <sup>3</sup> . About 18500 yBP.	Overhair, n = 10	280–294	Numerous, long, light reddish fragments	Fragmental, 4%	Cylindrical or slightly flattened (transversal section is ovoid or triangular)? With wide longitudinal groove on lower part of the shaft	
	Guard I, n = 5	197–203				
	Guard II, n = 5	136–142				
	Underhair I, n = 5	45–68	Numerous, short, light yellow fragments	Absent	Cylindrical or slightly flattened	
	Underhair II, n = 5	17–23				
Hind leg						
No. 7. "Yuka", Oyagogskiy ravine, Yakutia. Female, <i>subadultus</i> . No. 1. YuS <sup>4</sup> . 34300 (+260, -240) yBP (GrA-53289)	Overhair, n = 10	240–260	Numerous fragments with a base of the shaft, length 32 cm	Absent	Cylindrical	
Original data	Guard I, n = 10	136–147	Numerous long fragments			
Lower part of a trunk						
No. 8. The Mal'yi Lyakhovskiy Island, Yakutia. MM <sup>5</sup>	Overhair, n = 5	158–169				
	Guard I, n = 5	113–128	Rare, short, dark grey fragments	Absent	Cylindrical	
	Guard II, n = 5	56–79				
Forearm						
No. 9. X-probe – unknown species, woolly mammoth or woolly rhino. The Bol'shaya Chukoch'ya River. F-2362/1. MM	Overhair, n = 10	271–339	Numerous, short, red-brown (claret) fragments	Fragmental, 5% or 4 narrow veins, a medial vein is thickest	Cylindrical	
					Hair from an unknown part of the body	
	Overhair, n = 10	158–203	Light and dark brown fragments, length – 6.5–14.0 cm,	Fragmental, 5–21%, or absent	Cylindrical or slightly flattened and curved	

Table 1. (Continued)

Reference	Species, numbers of specimens, the name and place of storage. Sex and age. Collection number. Place of storage. Geological age	Topography of probes, categories of hair, n – number of hairs studied	Width of a shaft, lim, $\mu\text{m}$	Length, mm, stiffness, coloration of fragments	Width of the medulla, % of the shaft width, lim	Shaft configuration
Original data	No. 9. X-probe – unknown species, woolly mammoth or woolly rhino. The Bol'shaya Chukoch'ya River. F-2362/1. MM	Guard I, n = 5	113–136	Light yellow and white fragments, length – 4.5–11 cm	Fragmental, 20–50%	Cylindrical with longitudinal groove
		Guard II, n = 5	80–90	–	–	Cylindrical or flattened
		Underhair, n = 10	34–45	White fragments, length – 5.6–6.1 cm	Absent	Highly curved (4–5 waves)

<sup>1</sup>Diamond and Precious Metal Geology Institute of the Siberian Branch of the Russian Academy of Sciences, Yakutsk.

<sup>2</sup>Museum of Ust'-Lensky Nature Reserve.

<sup>3</sup>Academy of Sciences of Yakutia, Yakutsk.

<sup>4</sup>Yukagir Society, Yakutsk.

<sup>5</sup>Mammoth Museum, Yakutsk.

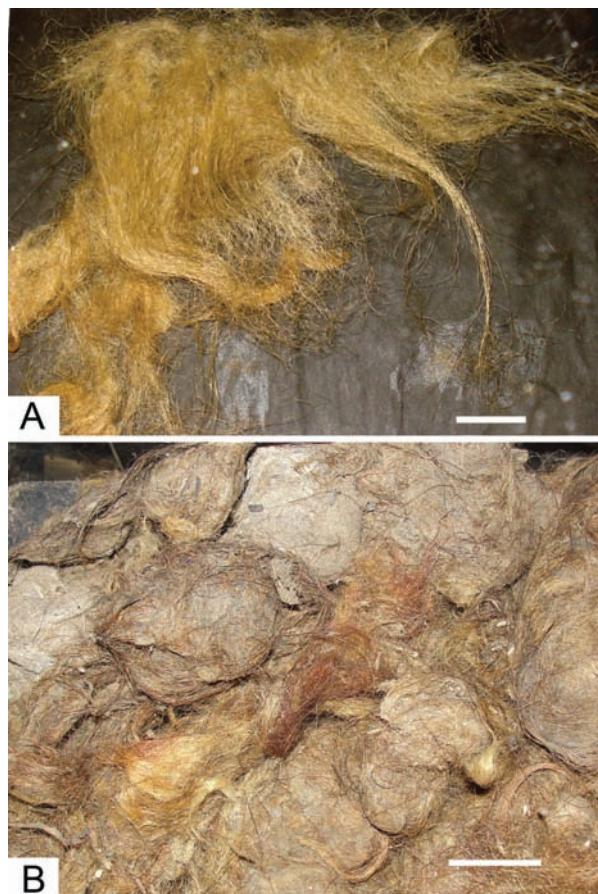


Fig. 2. The hair of the woolly rhino (probe No. 1) (A) and the matted hair (wads) of unknown species (woolly mammoth / woolly rhino?) (X-probe No. 9) (B). Microphoto. Scale bar = 1 cm.

loosely covered with reddish-brown hairs. The hair microstructure has been studied in the black rhinoceros *Diceros bicornis* (Linnaeus, 1758) (Table 1). Its hairs lack a medulla, but have a dense cortex, and the cuticle scales are tightly adjacent to the shaft and have a strongly incised apical edge (Ryder 1962). Eye lashes grow on the upper and lower lid (Van den Bergh 2009) (Table 1), whereas vibrissae are absent, although a genal vibrissa bud has been found in a black rhinoceros fetus (Cave and Wingstrand 1972). The reduction of hairs in recent living rhinoceros, as well as the absence of a subcutaneous adipose layer and the presence of large and actively secreting sweat apocrine glands, are related to the thermoregulation of these tropical animals. Likewise, the thick coat of the woolly rhino facilitated its adaptation to the harsh glacial climates.

In a recent paper, Tridico et al. (2014) concluded that the hairs of the woolly mammoth and woolly rhino are similar in colour, shaft shape, and other characters, and therefore gave a universal description of the hair of the two species. They confirm the tiered structure of the woolly mammoth hair, and, based on the study of its pigment distribution reject the popular myth that the woolly mammoths were red-haired, as they are shown in museum displays. Most likely, the coloration of the woolly mammoths was variegated, despite the popular belief that their wool was either from dark brown to black, or light coloured (the latter is supported by the molecular genetic evidence; Römpeler et al. 2006). It has been shown that the woolly mammoth and woolly rhinoceros hairs were similar in having "multiple medullae", and in resilience and variability (in our terminology differentiation of the hairs into categories and orders). These characters could have helped both species to survive the cold. However, the hair structure of the woolly mammoth and woolly rhino remains insufficiently studied, so the identification of these species based on hair microstructure is not yet developed. Therefore this study aims at recognizing species-level characters and adaptation of hair microstructure in these two members of the Pleistocene fauna, and raises the possibility of their identification from hair structure.

The results of morphological diagnostics of the hair tested were verified by genomic sequencing. Despite considerable interest in the woolly mammoths, the entire genome has not yet been reconstructed for these animals, and whole genome sequencing for the woolly rhino is not even on the horizon. Presently, sequences of a number of nuclear genes and the complete mitochondrial genomes are known for both species (Krause et al. 2006; Miller et al. 2008; Willerslev et al. 2009). In the absence of whole genome sequences of the species studied, it is possible to use the genomes of closely related species. In our case, we could only access the genomes of the African white rhinoceros and the African elephant. Thus, the analysis included two mitochondrial genomes, and the genome assemblies of two closely related species.

## MATERIAL AND METHODS

The studied hair specimens included two individuals of the woolly rhino (hereinafter 'rhinoceros'), and six individuals of the woolly mammoth (hereinafter 'mammoth'), found in various years in Yakutia

(Fig. 1) (see detailed descriptions of occurrences in Boeskorov et al. (2007, 2009, 2013); Boeskorov (2012)) and one individual not identified to genus (mammoth/rhinoceros?) X-probe No. 9 (Fig. 1, 2B) (Table 1). Hairs from the X-probe were chosen for genomic sequencing as they were the most difficult to identify by other means.

The hairs (number of hairs studied indicated in Table 1) were separated into categories and orders under the binocular microscope. This distinction was made by the thickness and configuration of the hair fragments' shafts. The hair and medullar thickness were measured under an "Amplival" light microscope (VEB Carl Zeiss, Jena), and "Leica DMLS" microscope with a digital video camera "Leica DMLS" (Germany) using a  $\times 10$  eyepiece and  $\times 10$ ,  $\times 40$ , and  $\times 63$  lenses. The largest of the overhairs and guard hairs were studied under JSM 840A (Japan) and "TESCAN" (Czech Republic) scanning electron microscopes. For the SEM study the hairs were washed and degreased in shampoo, then washed in distilled water, and dehydrated in alcohol of increasing concentrations. Longitudinal and cross sections were cut using a sharp razor blade and were fixed on stubs with clear nail polish. The samples were coated in an Edwards S-150 A (UK) gold sputter, then viewed and photographed in the accelerating voltage of 15 kV. Electronic grafts were made from longitudinal and cross sections of the base and the mid-shaft, and also from the cuticle surface along the shaft from the base to the mid-shaft and to the top. The electronic grafts were finalized using Adobe Photoshop CS2. Morphometric data were analyzed using the "STATISTICA 6.0" program.

Genetic studies were performed independently from morphological studies, with the results compared at the last stage of the analysis. The DNA extraction from the hair of the X-probe was performed in sterile conditions using modified modification of Yang et al.'s (1998) method. A sample of fleece was washed twice in alcohol and water. The fleece was then submerged in 10 ml of a solution containing 0.5M EDTA pH 8.0; 0.5% lauroylsarcosine sodium salt solution (Sigma) and 0.3 mg/ml proteinase K. The suspension was stirred for three hours at 55 °C, and the supernatant was removed after centrifuging (30 mins, at 15,500 g). The remaining fleece was submerged in 10 ml of the same solution and was placed in a mixer overnight at 55 °C. It was then centrifuged for 30 minutes at 10,000 g. The supernatant volume

was brought up to 250–300  $\mu$ l in the Amicon Ultra 5.000 (Millipore) concentrator at 10,000 g on the same rotor and was purified using the MinElute PCR Purification Kit (Qiagen). All the procedures followed the “Authenticity Protocol” (Hofreiter et al. 2001; Pääbo et al. 2004; Willerslev and Cooper 2005) in a separate sterile room taking all reasonable precautions to remove possible sources of contamination. A fragment library was prepared from the extracted DNA using the NEB Next Ultra Kit (NEB), excluding the DNA fragmentation stage. The sequencing was performed using a MiSeq (Illumina) genomic sequencer using the MiSeq Reagent Nano Kit v2 (Illumina).

The data were analyzed using CLC GW 7.0 (CLC Bio). Paired reads were filtered for quality (ambiguous limit = 2, quality limit = 0.031). The sequence mapping was performed using reference genomes of the African white rhinoceros (*cerSim1.0*, GenBank: AKZM00000000.1), African elephant (*loxAfr3.0*, GenBank: AAGU00000000.3), mitochondrial genomes of the rhinoceros (NC\_012681.1), and mammoth (NC\_007596.2) with the following parameters: mismatch cost = 2, insertion cost = 3, deletion cost = 3, length fraction = 0.8, similarity fraction = 0.9, global alignment = no.

## RESULTS AND DISCUSSION

**Hair differentiation.** In the mammoth and rhinoceros the hairs are well differentiated in a similar manner, and they cannot be distinguished by the number of categories and orders. On the body, the upper tier is composed of long, thick overhairs. The middle tier is composed of numerous guard hairs of two-three orders, whereas the innermost dense tier is composed of twisted downy hairs forming a thick underfur in some parts of the body. The hair length in the two species cannot be meaningfully compared, as complete hairs are only occasionally preserved, whereas hairs in the samples (including those studied) are generally broken and present only as fragments. It is known that the mammoth hair length could reach 1 m (Vereshchagin 1977). In various parts of the body of the Yuribei mammoth the length of the overhairs ranges from 9 cm on the side to 70 cm on the tail. The length decreases in the following order in the topographic row of probes: tail, forearm, thigh, side, groin, belly, and the back (Sokolov and Sumina 1982). The longest hairs were on the tail and the shortest in the

groin and on the back. These data cannot be meaningfully compared with the general pattern of fur in the mammoth. It is thought that in mammoths the structure of the fur and its tiering does not change with age, but the length and thickness of hairs is greater in adults (Sokolov and Sumina 1981a). It is clear that tiered structure of fur and very long overhairs and guard hairs, as well as dense underhairs, were the main adaptations to afford the animal a ‘puffier’ or bulkier appearance than the woolly mammoth whose hairs were not buckled. It is also thought that “buckled” hair shafts afforded the animal a bulkier appearance (Tridico et al. 2014). However, slight twisting or “buckling” of overhairs and guard hairs may be a result of post mortem deformation including processes in the permafrost. High density of the fur with a weakly developed medulla decreased the volume of the air layer in the fur, hence, decreasing its insulating properties. Therefore it is reasonable to suggest that in live animals, the mammoth and rhinoceros fur was fluffy rather than compact.

Measurements of hair thickness are the easiest to perform, even if the hairs are only partly preserved. Our measurements show that the maximum width of overhairs and guard I is definitely greater in mammoths than in rhinoceroses (in both cases  $p < 0.001$  following a Student’s t-test) (Fig. 3A), which can be used as a marker for distinguishing the hairs of these two species. In the X-probe this value does not exceed 203  $\mu$ m and is definitely less than in the mammoth ( $p < 0.001$ ), but similar to that of the rhinoceros. Comparison of the thickness of these hairs with those from the different parts of the body of the Yuribei mammoth (Sokolov and Sumina 1982) shows that they may have come from the belly. However, the discriminant function analysis of the total metric data (thickness of the overhairs and guard hairs, cuticle index of the overhairs, guard hairs and downy hairs) is ambiguous, and does not exclude the possibility of the hairs from the X-probe being from the rhinoceros (Fig. 3B, Suppl. 1).

**Hair coloration.** The colour of the mammoth overhairs and guard hairs studied ranges from light-reddish, dark- and reddish-brown to black (Table 1). Usually, guard hairs are lighter in colour than overhairs, while downy hairs (white, light-grey, yellowish) are lighter than guard hairs. In the woolly rhino, black, brown, yellowish-golden, and whitish hairs are also found, but no prominently reddish-brown hairs have been found. Certainly, natural hair coloration

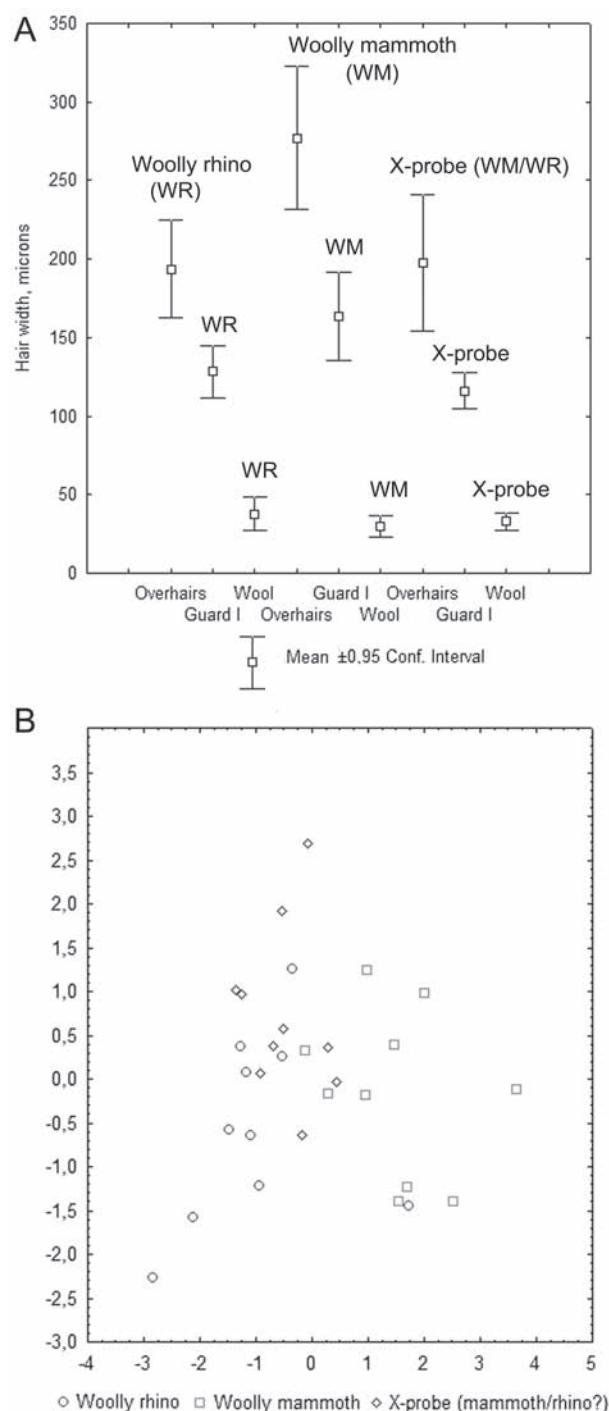
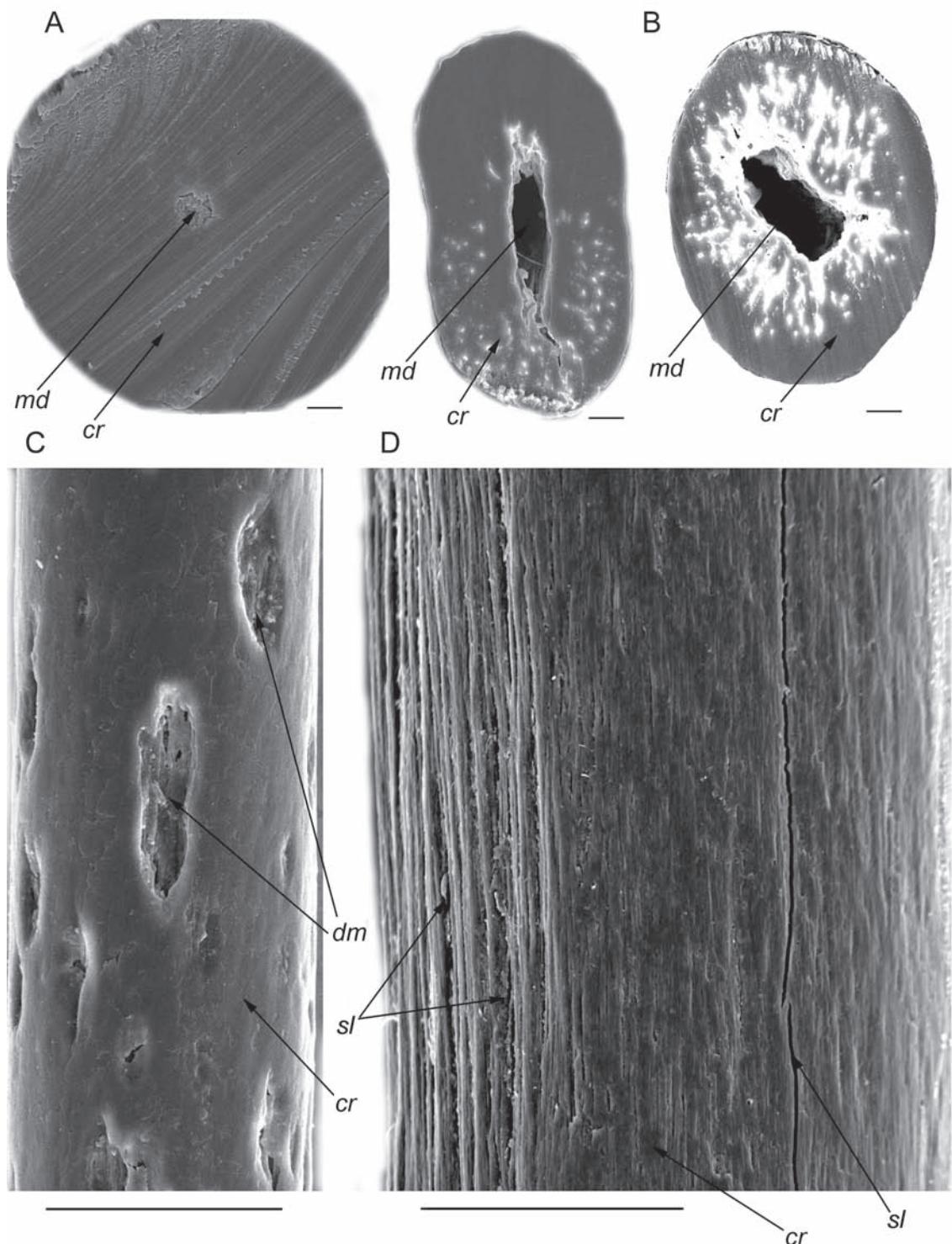


Fig. 3. Maximum thickness of hairs ( $n = 90$ ) of different categories (A) and the discriminant function analysis for the main metric data ( $n = 120$ ) (B) on hairs of the woolly mammoth (WM), woolly rhino (WR) and unknown X-probe (WM/WR?).

could have been altered taphonomically, but it is likely that the rhinoceros did not have reddish-brown hairs (as do some probes of the mammoth hairs). Tridico et al. (2014) suggested that the mammoth hair was mottled/variegated in appearance but did not have the vivid red/orange colour often depicted in museum displays. It should be said that some authors describe zonally coloured the mammoth overhairs: a black base, lighter-coloured tip, and a lighter middle (Sokolov and Sumina 1982). However, it is not possible to precisely estimate how natural these colours were, because during the long burial in the permafrost, hairs can not only lose natural coloration, but also accumulate pigment from the substrate. Using the molecular-genetic method, it was shown that the mammoths, like other mammals, had the MC1r gene, the expression of which controls polymorphism of hair coloration, from black and dark-brown to ginger or snow white (Römpl et al. 2006). Therefore it has been suggested that the mammoths could have been dark and light coloured. However if the differentiation of the mammoth fur is taken into account, it is logical to suggest that hairs of different types could be coloured differently in the same individual. For instance overhairs and guard hairs could be dark, and the underhairs could be light-coloured or vice versa. In addition, the mammoths could have had seasonal moulting, for example with the appearance of the light-coloured fur in winter. Individual variability in coloration was most certainly present as well. The hairs from the X-probe fits well into the general colour range of the mammoth and rhinoceros fur, but the absence of the reddish-brown hairs characteristic of the mammoths should be noted (Fig. 2B).

**Hair shaft configuration.** Configuration of the overhairs is usually cylindrical in both species and in the X-probe, but the mammoths more commonly had slightly flattened hairs (oval, triangular, or ovate in cross-section) and even with one narrow longitudinal groove in the lower part of the shaft, which agrees with the previously described hairs (Sokolov and Sumina 1981a) (Fig. 4A, B) (Table 1). Apparently, many hair shafts of both species became triangular, compressed, or ovate-shaped in cross-section, and also with longitudinal grooves, as result of strong mechanical compression. The shaft surface is strongly damaged (Fig. 4C, D) and even completely lost cuticle hence many large hairs resemble resilient fishing line. Their cortical layer has numerous longitudinal fissures (Fig. 4D). Guard hairs have a regular and



**Fig. 4.** An overhair transversal section of the woolly mammoth (WM) (A) and the same of two overhairs of the woolly rhino (WR) (B); the damaged surface of guard hair (C) of WR; the numerous deep longitudinal slits on an overhair (D) of WM: *md* – medullar cavity; *cr* – cortex; *dm* – damages of the shaft; *sl* – slit. SEM. Scale bars = 10 µm (A, B); 100 µm (C, D).

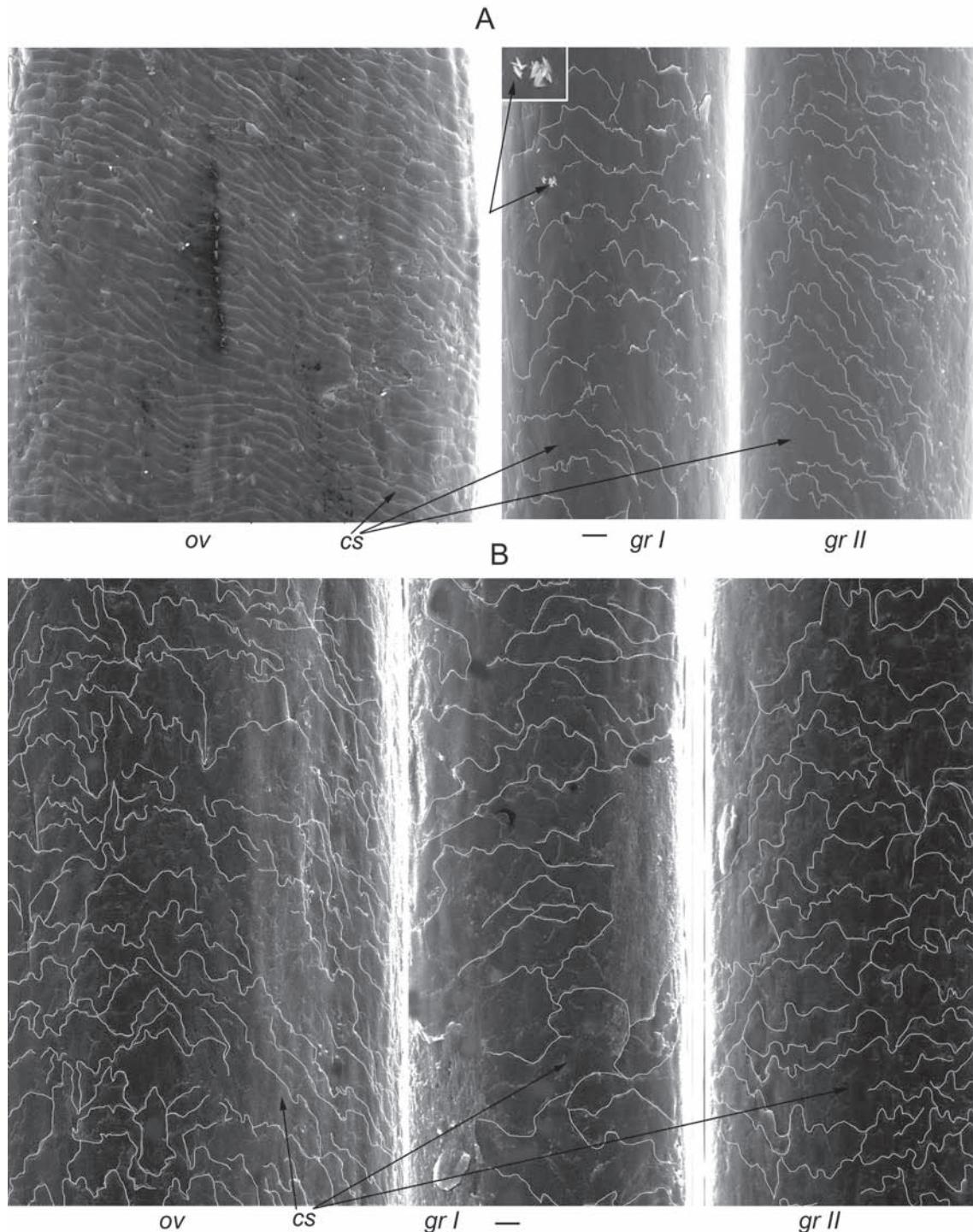
curved cylindrical or slightly flattened shaft. Downy hairs are long, unusually thick throughout the entire shaft length and variously twisted (Table 1).

**Cuticular (surface) scale pattern.** The cuticle of most studied hairs is strongly damaged and is preserved only in some areas of the shaft. It is semi-circular or circular (scales wrap up the shaft completely or half-way), variously strongly compressed in overhairs and guard hairs, with strongly incised free edges of the scales, many of which in the guard hairs are extremely high (20–35  $\mu\text{m}$ ) (Fig. 5). For comparison, in the black rhinoceros the cuticle is strongly flattened and is only 5–8  $\mu\text{m}$  thick (Ryder 1962). However, in large overhairs from the body of rhinoceros No 1, the cuticle is not very thick (up to 8–10  $\mu\text{m}$ ) and is at an angle of 30° to the cross axis of the shaft (Fig. 4A), although in guard hairs it is as thick as in the mammoth. In hairs from the X-probe, the scale height varies at the base and in the middle of the shaft and within one topographic area. The absolute maximum scale height is 22–30  $\mu\text{m}$  in the middle of an overhair and in the middle and at the base of guard I – 14–28  $\mu\text{m}$  and 40  $\mu\text{m}$ , respectively. It is possible that in the fossil species, large scales are revealed as a result of the destruction of the outer layers of the multilayered cuticle exposing deeper layers. However the undamaged cuticle of the guard hairs and downy hairs is also thick – 20–25 and 15–20  $\mu\text{m}$ , respectively in the mammoth and up to 35  $\mu\text{m}$  in the rhinoceros. Its free edges are undulating and weakly incised. The cuticle index (the maximum scale height to shaft diameter ratio) is definitely different in the mammoth and rhinoceros ( $p = 0.01$ ), especially in downy hair: 0.38–0.51 in the rhinoceros and 0.29–0.44 in the mammoth. However in X-probe, the cuticle index is not reliably different ( $p = 0.1$  in both cases) from such in these two species. Thus the specific assignment of the X-probe based on this character is not possible. In both species some high cuticle scales have a triangular apical area (Fig. 4). Many scales are oriented as 30–60° to the transverse axis of the shaft. At the base of the downy hairs, scales with a smoothed edge are oriented across the shaft, but higher up the shaft they are aligned at an angle of 45–50° to its transverse axis.

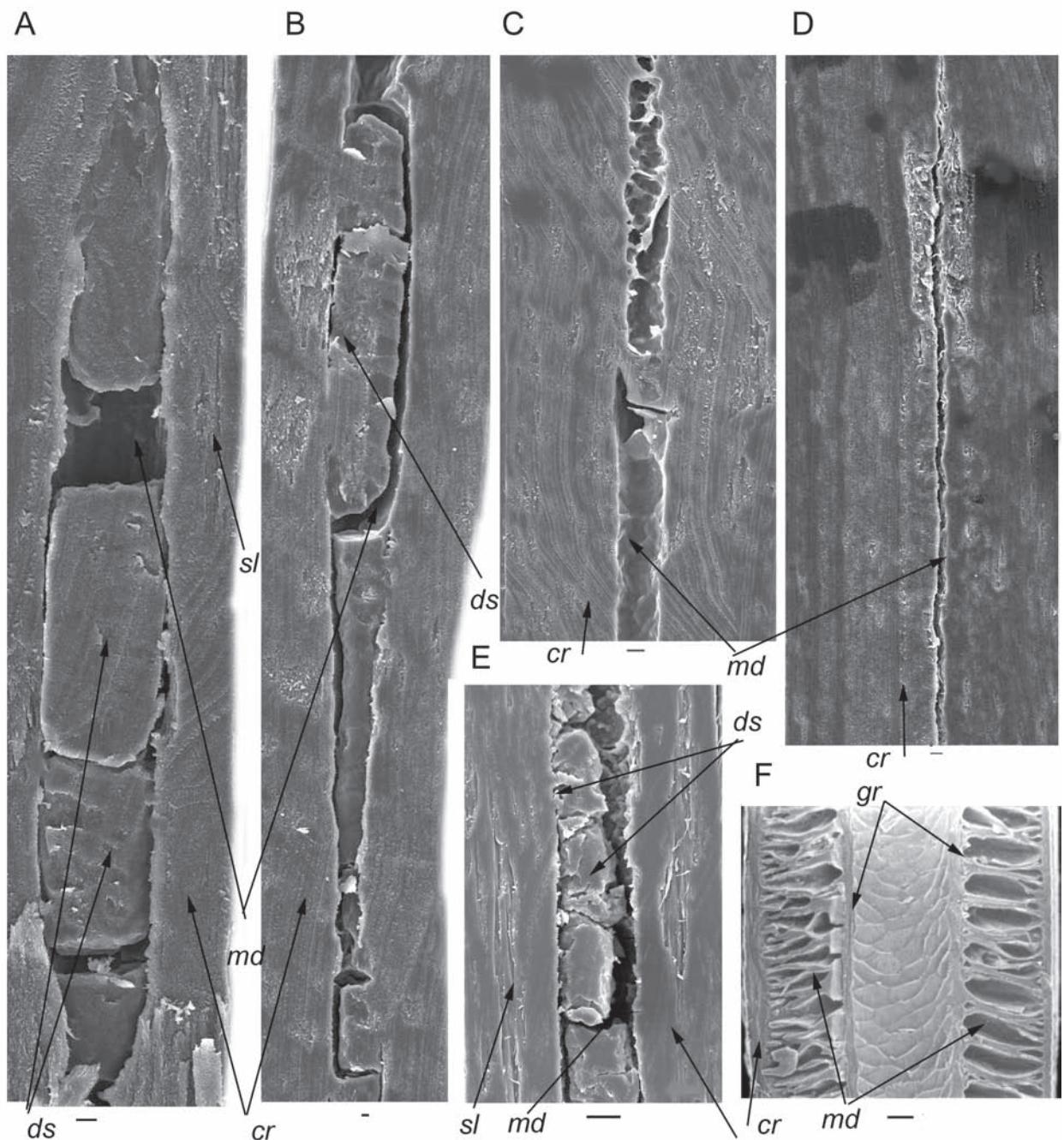
**Cortex structure.** The cortex layer in the hairs in the species studied is relatively dense, but as mentioned above, with many longitudinal slits, extending along the shaft (Fig. 4D). It is known that the slits appear (Hausman 1944) in the hair cortex: (1) when

it is still being formed in the hair follicle (“fusi”) and exist throughout the natural process of keratinization of the hair shaft, making the cortex more elastic and facilitating transport of tissue substances and gases; (2) as a result of longitudinal expanding of spaces between the keratinised cortex produced by mechanical compression (“fractile fusi”) (in this case probably by pressure of the frozen substrate). The slits are apparently not specific to particular species and are commonly found in other recent species (Chernova and Tselikova 2004; Chernova et al. 2011). In some cases of forensic practice, these structures have helped in identification of victims. The slits are readily discernible on the longitudinal and transverse sections of the shaft. They are more numerous, larger and longer (along the shaft) at the hair base and in coarser hairs. Under the light microscope they, like pigment granules, appear dark-coloured, and special methods exist for their differentiation (Hausman 1944). Apparently, some authors interpret these slits in the mammoth and rhinoceros hairs as medullar structures (see below) (Tridico et al. 2014).

**Medulla structure.** A medulla is found only in some large hairs in all three objects of study. It is difficult to observe under a light microscope because when hairs with a damaged cover are placed in any liquid medium (water, glycerine, xylene, etc.) the solution quickly penetrates into the unprotected and usually unpigmented medulla, so within seconds it becomes totally indiscernible. In the rhinoceros and X-probe, the interrupted medulla is only present in some overhairs and thick guard hairs (guard I), but if present then it is well-developed (Table 1) (Fig. 4B; 6A, B). For instance in the guard hairs of specimen No. 1, an irregular strand of fragmented medulla occupies up to half of the shaft thickness. In guard I of specimen No. 2 and X-probe the fragmentary medulla is also well-developed and at the base of the hair its strand is filled with large cylindrical discs divided by narrow air spaces. Nothing similar was observed in the mammoth hairs (Fig. 6C). Surprisingly, we described a similar structure in the vibrissae of a weasel *Mustela nivalis* L., 1766 (Mustelidae, Carnivora) (Fig. 6E) (Chernova and Kulikov 2011). In the rhinoceros, the maximum size of such a cylinder is 140 × 60  $\mu\text{m}$  with a guard hair thickness of 140  $\mu\text{m}$  (medulla is 43% of shaft thickness), and in the weasel it is 20 × 25; 80  $\mu\text{m}$  and 40%, respectively. The architectonics of the uniserial medulla as an alternation of compact keratinous cylindrical bodies and narrow air spaces,



**Fig. 5.** Cuticular patterns in the middle of the shaft of hairs of different categories of the woolly rhino (A) and the woolly mammoth (B): *cs* – cuticular scale; *ov* – overhair; *gr I* – guard I; *gr II* – guard II. Inset – foreign body (seeds?) on the shaft, indicated by arrows. Drawing on electronic grafts. SEM. Scale bars = 10 µm (A, B).



**Fig. 6.** Microstructure of the medulla of the overhairs of the woolly rhino (A), X-probe (woolly mammoth/woolly rhino?) (B, C), woolly mammoth (D), of a vibrissa of the weasel *Mustela nivalis* (E), and the double medulla of a spine of the spiny rat *Maxomys surifer*. Longitudinal sections of middle of the shafts. Symbols as shown in Fig. 4. Additional symbols: *ds* – medulla cylindrical disc; *gr* – longitudinal groove, lines with cuticle; SEM. Scale bars = 10  $\mu$ m.

**Table 2.** Results of the hair DNA analysis of recent species of elephants and rhinos, and extinct woolly mammoth and woolly rhino.

Reference	Mapped reads	Genome fragment covered by reads	Mean coverage
<i>Loxodonta africana</i> (scaffold)	2.96%	0.007%	$1.3 \times 10^{-4}$
<i>Ceratotherium simum</i> (scaffold)	0.13%	0.0006%	$1.3 \times 10^{-5}$
<i>Mammuthus primigenius</i> (mitochondrial)	0.07%	45%	1.21
<i>Coelodonta antiquitatis</i> (mitochondrial)	0.003%	2%	0.042

certainly improves the strength of the shaft and effectively protects it from rough mechanical impacts, such as contact of the vibrissae and the substrate in the weasel or fur (in this case of the rhinoceros leg) with rough vegetation or a hard snow crust. In the mammoth, the hair medulla is degenerative, representing an irregular (sometimes moniliform) narrow, continuous or interrupted strand, extending in the middle of the shaft (Fig. 6D). It usually occupies from 5% to 15% of the shaft thickness (Table 1). The inner structure of the medulla is usually destroyed and details of its architectonics are indiscernible.

Thus, the rhinoceros and mammoth hairs can be distinguished using the degree of the development and architectonics of the medulla present in some large hairs. X-probe contained hairs, which, using this characters can be assigned to the mammoth and rhinoceros (Fig. 6A, C, D). In addition, a comparative analysis (Chernova and Kirillova 2013) of the probe showed hairs of the ancient bison *Bison* spp., with a well-developed lattice-like medulla.

**DNA control of the X-probe hair.** We originally thought that the wool in the X-probe came from either the rhinoceros or mammoth. Because the ancient DNA is highly fragmented, we used low-coverage full genome sequencing that should have allowed us to identify the species to which the hairs belonged. DNA was extracted and a fragmentation library was prepared, this was then sequenced using a MiSeq platform which yielded a total of around 40 Mb of  $2 \times 250$  bp paired-end reads. *Mapping of the paired-end reads* was performed with a high degree of matching to the *reference* genome (at least 72%). As references were used mitochondrial genomes of the rhinoceros and mammoth, and the genome assemblies of the African white rhinoceros and African elephant. For the African white rhinoceros the mapped reads accounted for 0.13% of the total reads, while for the African elephant this was 2.96% (Table 2). Similar results can be observed in the case of mitochondrial

DNA. These data suggest that when genomes related to that of the mammoth are used, successful mapping is always 10–20 times higher than when mapped to comparably related genomes of the woolly rhino. These results support the assignment of the tested hairs to the mammoth.

**On hair “multiple medulla”.** Multiple medullar-like structures in the mammoth / rhinoceros hairs consist of several (up to four) continuous or interrupted very narrow parallel longitudinal lines, aligned along the shaft (Tridico et al. 2014). It is also found in the hairs of the African elephant foot and hairs from the back and head of the Indian elephant (ibid.). In the mammoth, a wider central strand and narrow lateral strands are aligned to the base of large hairs (Valente 1983). In humans, paired medullae are extremely rarely found in moustache, beard, and chest hairs (Hicks 1977; Ogle and Fox 1999; Pavlov 2000a, b; Deedrick and Koch 2004). Sometimes multiple medullae are present as narrow parallel fiber strands between the cortical cells (Ogle and Fox 1999). Such a medulla does not have a diagnostic significance. Under the light microscope at the base of the singular overhairs of the right leg of the mammoth No. 8 we observed three-four short longitudinal very narrow pigmented parallel structures (“cords”), of which only the central had thickness usual for the mammoth hair medulla, whereas other cords are present as narrow layers between the layers of cortical cells. SEM observation did not reveal such a medulla.

We agree with Gilbert et al. (2007) that large clusters of pigment, remains of nuclei, ovoid bodies, or as mentioned large slits usually present between cortical cells. Apart from the longitudinal slits, the cortex does indeed contain large clusters of pigment, for example in the hairs of the mane of the Dyukar mummy of the Pleistocene horse *Equus lenensis* Rusanov, 1968 (Chernova et al. 2011). Here, the pattern is the same as in the cross section of the mammoth/ rhinoceros hair. Keratinous cortical “cells” and

their large dark wrinkled nuclei are visible. Apparently, in damaged hairs of fossil species, numerous longitudinal fissures and external damages are filled with air, substrate, fungi, algae, etc. Under the light microscope they are observed as pigmented medullar structures. If multiple medullae are indeed present, they would be so degenerated in the mammoth and rhinoceros that they could never improve insulation as suggested by the authors (Tridico et al. 2014).

These erroneous interpretations do not mean that true “multiple” medullae are absent from mammal hairs. For instance, the extinct giant sloth *Nothrotherium shastense* Hoffstetter, 1954 (Hausman 1944), which survived the last glaciation, had a typical “paired medulla”, which looks like two parallel longitudinal cords with cellular architectonics. In recent mammals, such medullae are also observed, formed when a deep, wide groove extends along the central area of the shaft containing no medulla and separating the medullar cord into two lateral longitudinal bands (possibly also present in the giant sloth hairs). This groove is also visible in SEM, and under the light microscope, in the intact hairs it is invisible and its presence can only be identified from the shape of the cross-section. Paired medullae are present in the spines of many tropical Muridae – *Tokudaia osimensis* Abe, 1934, *Maxomys surifer* Miller, 1900 (Fig. 6F), *Niviventer confucianus* Milne-Edwards, 1871, *N. cremoriventer* Miller, 1900, *N. fulvescens* Gray, 1847, *Acomys cahirinus* É. Geoffrey, 1803. In porcupines (*Trichys fasciculata* Shaw, 1801, *Erethizon dorsatum* L., 1758), medullae can be considered triple, as three cords are separated by septa of cortex. Setae of the peccary *Pecari tajacu* L., 1758 (Tayassuidae Palmer, 1897) have medullae with five cords. Hedgehog spines (*Echinaceus* L., 1758) also have complex medullae (Chernova and Tselikova 2004).

## CONCLUSION

In the mammoth and rhinoceros, the triple hair coat structure is relatively well and similarly differentiated, as this feature was the main adaptation of the wool of these species to harsh climates. On the body, the upper tier is composed of sparse long and short overhairs. The middle tier, of numerous guard hairs (or intermediate hairs) of two-three orders, whereas the lower dense tier is represented by thin weakly twisted downy hairs. The maximum thickness of the overhairs and guard hairs is certainly greater in

the mammoth than in the rhinoceros, and can be used for distinguishing these taxa. Coloration of hairs of different categories is similar in both species, except for the presence of red-brownish hairs in some areas of the mammoth body. The configuration of hairs of different categories is similar in both species, which include slightly compressed overhairs with a longitudinal groove at the shaft base.

Indeed, as it has been shown previously (Tridico et al. 2014) the cuticle and cortex of hairs are similar in both species and not specific to particular species. The cortex has many slits between cortical cells aligned along the shaft. The main interspecific differences in the hair microstructure are in the more developed medullar layer and its architectonics compared to the mammoth. The architectonics of the medulla is the main diagnostic character in the identification of these species. In the rhinoceros it is identical to the vibrissae structure of some small mammal predators and is an adaptation to improve hair strength. Generally in both species, the medulla is degenerative and does not affect the insulation properties of the hairs.

The above data shows that the wads of the X-probe contained numerous mammoth hairs (confirmed by the DNA sequencing) and a few woolly rhino hairs. Additionally, the wads contained isolated hairs of an extinct bison species. The main diagnostic features distinguishing hairs of these species include shaft thickness and configuration, cuticle ornamentation, development and architectonics of the medulla. It can be suggested that these wads were a product of simultaneous burial of different animals at the same site (e.g., in a natural trap) or accumulation of carcasses brought together by streams or some other agents, but new research is needed to adequately interpret the taphonomy of this fossil site.

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**Supplement 1.** The metric data for the discriminant function analysis given on Fig. 3B.

No, hairs	Species	Width, $\mu\text{m}$			Index of the cuticle*	
		Overhair	Guard I	Downy hair	Overhair	Downy hair
1	Woolly rhino	158	135	23	0.34	0.57
2		250	125	34	0.29	0.51
3		158	113	56	0.26	0.57
4		226	117	23	0.25	0.45
5		146	124	34	0.31	0.32
6		200	125	45	0.29	0.38
7		169	125	17	0.25	0.35
8		158	113	56	0.18	0.34
9		200	113	56	0.32	0.32
10		271	192	34	0.16	0.44
11	Woolly mammoth	399	180	34	0.17	0.29
12		200	250	11	0.21	0.39
13		320	180	34	0.28	0.36
14		282	142	45	0.2	0.33
15		226	147	23	0.22	0.44
16		248	203	34	0.26	0.34
17		294	147	34	0.23	0.29
18		260	128	23	0.2	0.33
19		339	135	23	0.19	0.34
20		203	124	34	0.13	0.44
21	X-PROBE	305	113	34	0.24	0.5
22		226	136	23	0.2	0.42
23		305	124	34	0.28	0.37
24		150	113	23	0.25	0.27
25		158	124	45	0.16	0.33
26		169	90	34	0.1	0.27
27		158	113	23	0.33	0.33
28		158	124	45	0.18	0.34
29		192	90	34	0.28	0.35
30		158	135	34	0.29	0.34

\* Index of the cuticle is the ratio of height of the cuticular scale to the thickness of the hair in place of its location.